Supplementary Data

Selection of Virtual Screening Hits for Experimental Testing

The compounds from the virtual screen were chosen as follows:

i. The compounds ranking 1-20 from the screen to structure with PDB code: 1t6b were chosen for experimental testing.
ii. 17 of the top 150 scoring compounds from the screen to structure with PDB code 1tzo were chosen by eye based on their predicted interaction with E515 and K215 in the ligand binding pocket. Chemical clustering was used to ensure a diverse set of compounds was chosen.
iii. 5 compounds were selected that were common in both screening hitlists (1t6b and 1tzo) but not selected in i or ii.

The ranking of the lead compounds in the hitlist from the screen to 1t6b and 1tzo are summarized in Supplementary Table 1.

	Rank in screen against PDB 1t6b	Rank in screen against PDB 1tzo
5181401	4	-
5181385	10	70
5117235	16	-
5180717	53	198

Supplementary Table 1: The ranking in the hitlist for each lead compound is shown. The active compounds ranked higher in the screen against the monomer PA structure 1t6b

Purity of compounds

We requested data verifying the purity of the compounds that we purchased from ChemBridge Corporation, San Diego, CA. They provided us with spec sheets from a Nuclear Magnetic Resonance ^{1}H PMR spectra. Spectra are attached for the three compounds examined in this report. Compound 5181385 became unavailable after our initial tests, and purity data is not included for it. The company stated that these spectra demonstrated >95% purity (email attached).

However, since spectral data is not accepted as sufficient proof of purity, we tested the compounds ourselves using Reverse Phase HPLC (see attachments). To do this, we used a Waters 2695 Separation Module equipped with 2996 PDA detector. Analysis of chromatograms was done with *Empower* Software.

Leppla, Stephen (NIH/NIAID) [E]

From: Chembridge Support <support@chembridge.com>
Sent: Friday, April 27, 2012 12:42 PM
To: Wein, Alexander (NIH/NIAID) [F]
Cc: Leppla, Stephen (NIH/NIAID) [E]
Subject: RE: Certificate of Analysis

Dear Alex,

According to our NMR spectra (attached), all 4 compounds are >=95% pure.

Please note that there is apparently an error in the structure of ID 5117235 in our database. It is actually a free base, not a dihydrate. This should be fixed in the database soon.

If you have any questions, please feel free to contact me.

Sincerely,
Max Strongin
Manager, Customer Service
ChemBridge Corporation
11199 Sorrento Valley Rd., Ste 206
San Diego, CA 92121
Toll Free: (800) 964-6143 ext. 181
Phone: (858) 451-7400 ext. 181
Fax: (858) 451-7401

From: Wein, Alexander (NIH/NIAID) [F] [mailto:alexander.wein@nih.gov]
Sent: Thursday, April 26, 2012 2:39 PM
To: Chembridge Support
Cc: Leppla, Stephen (NIH/NIAID) [E]
Subject: Certificate of Analysis

Hello,

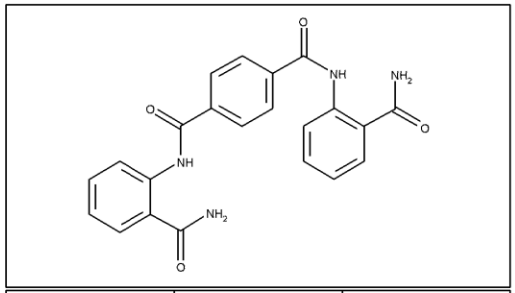
We purchased several small molecules (#'s 5117235, 5180717, 5181385, and 5181401) from your company and are trying to publish the results we obtained with these compounds. The journal requires proof of >95 % purity of all chemicals used in the manuscript, as well as 1H NMR and MS results. Does your company maintain records of this type or will we have to confirm the purity and identity of the purchased compounds?

Thanks,

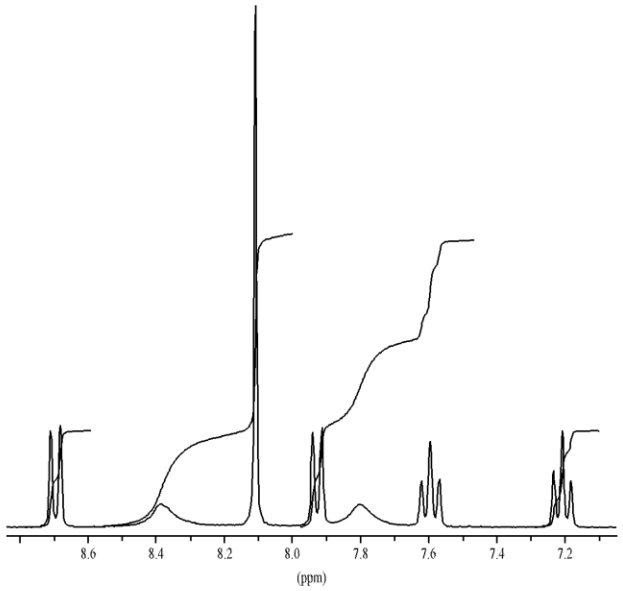
Alex

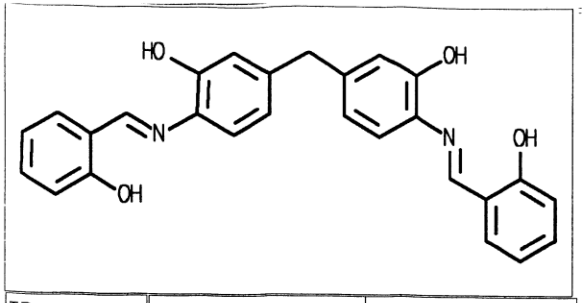
Alexander Wein
Microbial Pathogenesis Section
Laboratory of Parasitic Diseases
National Institute of Allergy and Infectious Diseases

/XXXX 181401X3.001



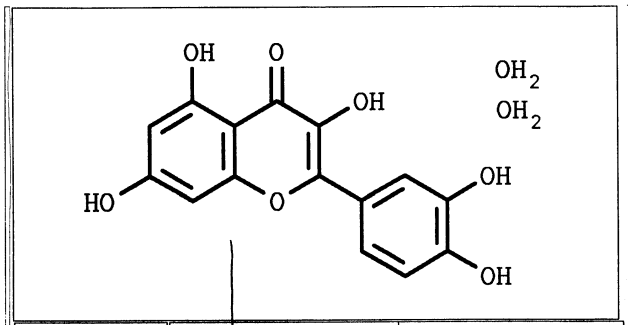
ID	181401	402.4132	$C_{22}H_{18}N_4O_4$





ID 80717	C27 H22 N2 O4	438.49

F=300.13 MHz (H-1) SI=16384 SW=4807.692 C1=7000 PW=1.0 RD=2.0 AG=1.491 NS=16 SR=4788.341 11 April 1996 SOLV DMSO

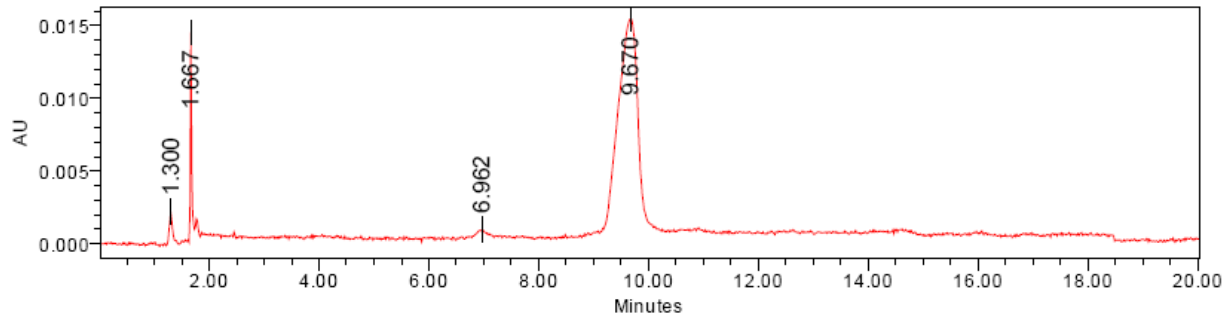


ID117235	C15 H14 O9	338.27

Analysis of N,N'-bis[2-(aminocarbonyl)phenyl]terephthalamide (Chembridge #5181401)

Method:

Column Agilent Eclipse XDB-C18, 150mm x 4.6mm I.D., 5µm particles
Mobile phase A: water with 0.1% acetic acid; B: acetonitrile
Gradient B: 0-30%, 20 min
Detection UV at 280nm
Injection: 5µl 10mM DMSO solution



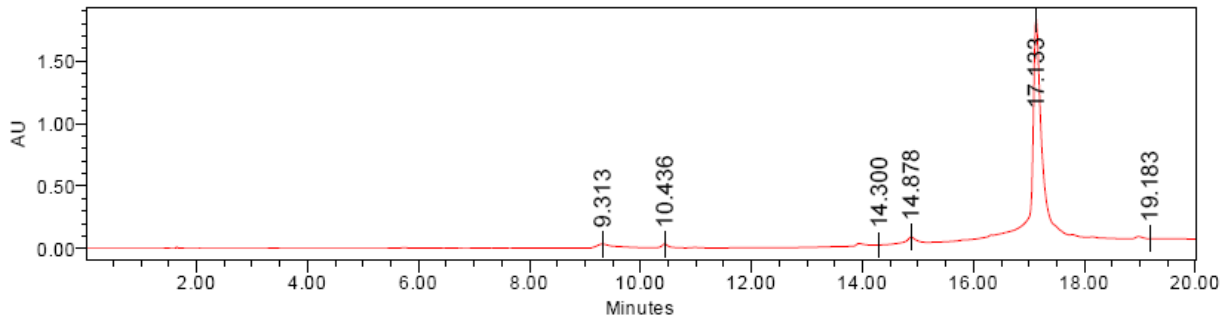
Peak Results

	Name	RT	Area	% Area
1		1.300	1384	0.34
2		1.667	10963	2.72
3		6.962	1322	0.33
4	5181401	9.670	390050	96.61

Analysis of 3,3'-methylenebis{6-[(2-hydroxybenzylidene)amino]phenol} (Chembridge #5180717)

Method:

Column Agilent Eclipse XDB-C18, 150mm x 4.6mm I.D., 5µm particles
Mobile phase A: water; B: acetonitrile
Gradient B: 30-100%, 20 min
Flow rate 1 ml/min.
Detection UV at 280nm
Injection: 5µl 10mM DMSO solution.



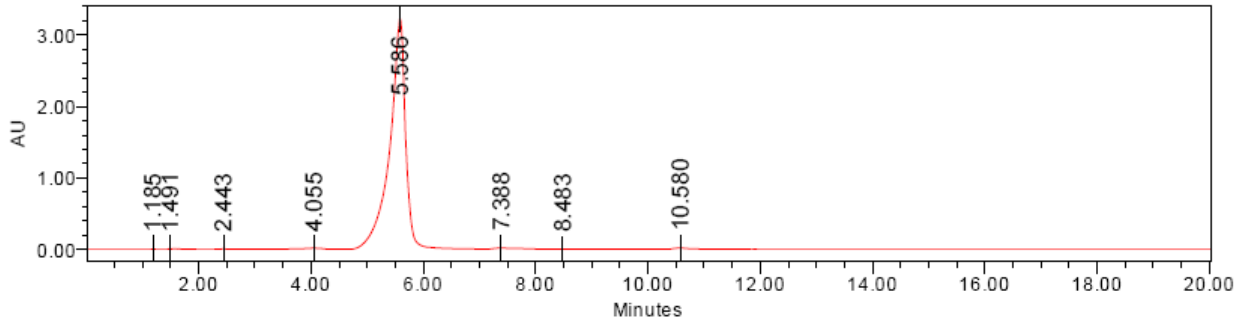
Peak Results

	Name	RT	Area	% Area
1		9.313	398149	1.42
2		10.436	284104	1.01
3		14.300	146475	0.52
4		14.878	486755	1.73
5	5180717	17.133	26750742	95.17
6		19.183	42943	0.15

Analysis of 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one dehydrate (Chembridge #5117235)

Method:

Column Agilent Eclipse XDB-C18, 150mm x 4.6mm I.D., 5µm particles
Mobile phase A: water; B: acetonitrile
Gradient B: 0-30%, 20 min
Flow rate 1 ml/min
Detection UV at 260nm
Injection: 5µl 10mM DMSO solution



Peak Results

	Name	RT	Area	% Area
1		1.185	36448	0.06
2		1.491	191520	0.29
3		2.443	162490	0.25
4		4.055	736997	1.12
5	5117235	5.586	63718572	96.54
6		7.388	697311	1.06
7		8.483	14250	0.02
8		10.580	445741	0.68